# A New Species of the Genus *Stylactaria* (Cnidaria, Hydrozoa) from Hokkaido, Japan

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ABSTRACT—A new species of athecate hydroid, Stylactaria multigranosi sp. nov., is described based on specimens from Hokkaido, Japan. Of 416 colonies collected, all of the mature ones (N=306) were female. Hydroids of S. multigranosi were associated exclusively with a gastropod, Nassarius multigranosus (Dunker, 1847), living on sandy-mud bottoms. This new species is characterized by having fixed eumedusoid gonophores without marginal tentacles. Eggs in S. multigranosi develop into planulae within the gonophores. Four types of zooids (gastrozooids, normal gonozooids, non-hypostomed gonozooids and tentaculozooids) were discovered. The taxonomy of Stylactaria is briefly discussed, with particular reference to morphology of gonophores and intercolonial variation in zooid polymorphism.

#### INTRODUCTION

Calder [1] proposed that *Stylactaria* Stechow, 1921 [2] should be adopted as the valid name for the nominal genus sometimes known as *Stylactis* Allman, 1864 [3] and assigned eight athecate hydroid species to the genus. *Stylactaria*, a genus in the polymorphic hydrozoan family Hydractiniidae, is characterized by having hydrorhizal stolons covered with perisarc.

Recently, two species newly assigned to Stylactaria, S. conchicola and S. uchidai, were redescribed from Hokkaido, Japan, after a detailed one year study [4, 5]. During the research, an unknown immature hydroid colony was discovered on a shell of a living gastropod, Nassarius multigranosus (Dunker, 1847), collected at a depth of 2 m by a bottom sampler from sandy-mud bottoms in Oshoro, Hokkaido. Subsequent study of many specimens collected from shells of the same gastropod species confirmed that the hydroid is a new species of Stylactaria. The present paper describes this new species following methods established in redescriptions of the two Stylactaria species from Hokkaido mentioned above [4, 5].

Accepted April 2, 1991 Received February 20, 1991

#### **MATERIALS AND METHODS**

A total of 416 colonies of Stylactaria multigranosi sp. nov., were collected from May to August, 1990 at Oshoro (43°12′N, 140°52′E) and from June to July, 1990 at Usu (42°31′N, 140°47′E), Hokkaido, Japan, where the water temperature varied from 11.3°C to 24.4°C. All colonies were found on living shells of a species of gastropod, Nassarius multigranosus (Table 7). These gastropods were collected by a bottom sampler from the sandy-mud bottoms, 1–5 m depth, or attracted to bait (dead fish) set on sandy-mud bottoms and rocky shores and scooped up with a net.

All of the mature colonies examined (306 out of 416 specimens collected) were female. They were kept in the laboratory without food at 12°C or 20°C in filtered sea water transported from Oshoro. The zooid composition of mature colonies was examined (Table 3).

The dimensions of zooids were measured under a binocular microscope in forty mature colonies (twenty specimens each from Oshoro and Usu) within a week after collection. In order to get fully extended zooids, colonies were anesthetized prior to measurement by dropping 10% MgCl<sub>2</sub> solution into the cultures. Thirty planulae, hatched from three colonies (type material) in the laboratory, were measured. Measurements of undischarged

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nematocysts in gastrozooids and larvae of holotype were taken using a microscope with phase-contrast optics.

Type specimens were not fed *Artemia* nauplii until their zooids and larvae were measured. After feeding on *Artemia* nauplii, the inner structure of gonophores became more apparent because the canals were reddish due to food particles. Type material was subsequently fixed with Bouin's fluid for an hour and sectioned to examine the inner structures of gonozooids and gonophores. Stolons were measured after being detached from the decalcified surface of shells.

Type material has been deposited in the Zoological Institute, Faculty of Science, Hokkaido University (ZIHU).

#### DESCRIPTION

Family Hydractiniidae L. Agassiz, 1862 Genus Stylactaria Stechow, 1921 Stylactaria multigranosi sp. nov. (Figs. 1-3) Type material. Holotype: ZIHU 495, female colony with gastrozooids and normal gonozooids, 2 m depth, Oshoro Bay, Hokkaido, Japan, 17. viii. 1990. Paratypes: ZIHU 496, female colony with gastrozooids, normal gonozooids and a nonhypostomed gonozooid, 3 m depth, Oshoro Bay, 18. vi. 1990; ZIHU 497, female colony with gastrozooids, normal gonozooids and a tentaculozooid, 1 m depth, Usu, Hokkaido, 25. vi. 1990. All type material collected by H. Namikawa.

Measurements of zooids, larvae and stolons of type material as in Table 1.

Etymology. The specific name multigranosi is derived from the species name of the host gastropod, Nassarius multigranosus.

#### 1. Zooids

Colony composed of four zooid types: gastro-zooids, normal gonozooids, non-hypostomed gonozooids, and tentaculozooids. Measurements of each zooid type are given in Table 2.

Gastrozooids (Fig. 1A): Monomorphic, cylin-

TABLE 1. Stylactaria multigranosi sp. nov. Measurements (mean ± S.D., range) of type material

	Gas (n=5)	Gon (n=5)	NhG (n=1)	Tz (n=1)	Lar (n=10)	$     \text{St} \\     (n=10)^{1)} $
Maximum length of bodies (mm)	$1.09 \pm 0.06$ (1.00-1.15)	1.10±0.15 (0.85−1.30)	0.50	4.20	$0.50 \pm 0.08$ (0.35-0.65)	
Width (mm)	$0.16\pm0.01 \ (0.15-0.18)^{2}$	$0.09 \pm 0.01  (0.07 - 0.10)^{2}$	$0.10^{3}$	$0.05^{4)}$	$0.08 \pm 0.03 \ (0.06 - 0.10)^{5}$	$0.83 \pm 0.15$ (0.63-1.13)
		$0.10 \pm 0.01  (0.10 - 0.12)^{3}$				
Height of hypostomes (mm)	$0.24 \pm 0.02$ (0.20-0.25)	$0.10\pm0.02 \ (0.08-0.11)$				
No. of tentacles	$9.6 \pm 0.5 \ (9-10)$	$6.8 \pm 1.0 $ $(5-8)$				
Length of long tentacles (mm)	$1.04 \pm 0.06$ (0.95 – 1.10)	$0.52 \pm 0.07$ (0.45 - 0.65)				
Length of short tentacles (mm)	$0.53\pm0.07$ (0.45-0.65)	$0.26 \pm 0.04$ (0.20-0.30)				
No. of gonophores		$5.2 \pm 1.4 $ (4-8)	1			
Diameter of gonophores		$0.31 \pm 0.02$ (0.25 - 0.35)	0.25			
No. of eggs or larvae		$3.6 \pm 0.7$ $(2-4)$	4			

Gas: gastrozooids; Gon: normal gonozooids; NhG: a non-hypostomed gonozooid; Tz: a tentaculozooid; Lar: larvae; St: stolon. All measurements are for holotype except NhG (for Paratype ZIHU 496) and Tz (for Paratype ZIHU 497). 1): measured points of stolon; 2): width at the circlet of tentacles; 3) width at the body portion with gonophores; 4): width at basal part; 5) maximum width of larvae.

Table 2.	Stylactaria multigranosi sp. nov. Measurements (mean ± S.D., range) of each type of zooid, as
well	as larvae and stolons of 40 specimens including type material

No. of colonies	Gas (n=200) 40	Gon (n=200) 40	NhG (n=15) 15	Tz (n=2) 2	Lar (n=30)	$ \begin{array}{c} \text{St} \\ \text{(n=30)} \\ 3 \end{array} $
Maximum length of bodies (mm)	$1.13 \pm 0.29$ (0.50-2.15)	$\begin{array}{c} 1.08 \pm 0.32 \\ (0.50 - 2.00) \end{array}$	$0.70 \pm 0.21$ (0.10-1.25)	3.90±0.30 (3.6-4.2)	$0.51 \pm 0.88$ (0.30-0.65)	
Width (mm)	$0.15 \pm 0.04$ (0.10-0.25)	$0.08 \pm 0.01 \ (0.05 - 0.10)$	$0.10\pm0.01$ (0.08-0.12)	$0.04 \pm 0.01$ (0.03-0.05)	$0.08 \pm 0.01$ (0.06-0.10)	$0.87 \pm 0.16$ (0.63-1.25)
		$0.09 \pm 0.02 \ (0.07 - 0.15)$				
Height of hypostomes (mm)	$0.23 \pm 0.03$ (0.15-0.30)	$0.12\pm0.03$ (0.05-0.15)				
No. of tentacles	$11.0 \pm 1.9 \ (8-16)$	$7.3 \pm 1.6 \ (4-11)$				
Length of long tentacles (mm)	$1.24 \pm 0.22$ (0.75-1.85)	$0.59 \pm 0.19$ (0.20-1.00)				
Length of short tentacles (mm)	$0.63 \pm 0.17$ (0.30-1.15)	$0.27 \pm 0.11$ (0.10-0.50)				
No. of gonophores		$5.0 \pm 1.5 \ (1-8)$	$1.3 \pm 0.5 \ (1-2)$			
Diameter of gonophores		$0.31\pm0.03 \ (0.25-0.35)$	$0.31\pm0.03 \ (0.25-0.31)$			
No. of eggs or larvae		3.9 ±0.9 (1-6)	4.4 ±0.5 (4-5)	···		

Abbreviations and measured positions in width as in Table 1.

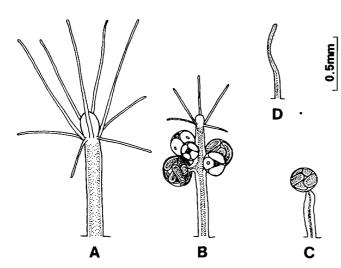


Fig. 1. Stylactaria multigranosi sp. nov. Zooids. A: gastrozooid (Holotype); B: female normal gonozooid with six gonophores in two whorls (Holotype); C: female non-hypostomed gonozooid bearing a gonophore on the top of body (Paratype ZIHU 496.); D: somewhat elongated tentaculozooid (Paratype ZIHU 497.).

drical and whitish, with blunt conical hypostome. Two types of filiform tentacles (short and long) arranged in a single circlet. Long tentacles almost as long as the body, projecting upwards and paralleling the long axis of body. Short tentacles about 1/2 as long as long ones, extending obliquely outward.

Gonozooids: Dimorphic, whitish.

Normal gonozooids (Fig. 1B) slender with blunt conical hypostome. Mouth at the center of hypostome. Two types of filiform tentacles (long and short) arranged in a single circlet around the base of hypostome, shorter than those of gastrozooids. Long tentacles projecting obliquely outwards. Short tentacles about 1/2 as long as long ones, extending horizontally outwards. Gastric cavity extending from mouth to the basal portion of body. Gonophores located about 1/4 to 1/2 body length below the tentacle whorl; arranged in one whorl (when there are four or less gonophores in a zooid) or two whorls (when there are more than four gonophores in a zooid), each whorl bearing one to four gonophores.

Non-hypostomed gonozooids (Fig. 1C) with neither hypostome nor tentacles, shorter than normal gonozooids. Gonophores located in a single whorl at distal end body. Non-hypostomed gonozooids present in 19% of the colonies collected

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TABLE 3. Stylactaria multigranosi sp. nov. Zooid constitution in 306 colonies examined

Zooid constitution	No. of colonies (%)			
Gas+Gon	245	(80.1)		
Gas+Gon+NhG	59	(19.3)		
Gas + Gon + Tz	2	(0.7)		
Gas + Gon + NhG + Tz	0	(0.0)		

Abbreviations of zooid type as in Table 1.

(Table 3).

Tentaculozooids (Fig. 1D): Monomorphic, long, slender, filamentous. Swollen distal end packed with nematocysts. Tentaculozooids occurred in 0.7% (two out of 306) of the colonies collected (Table 3).

## 2. Gonophores and larvae

Gonophores and larvae of both types of gonozooids similar in morphology and dimensions. Female gonophores only known. Each gonophore arising from a short stalk, spherical eumedusoid, with three or four radial canals and a ring canal; marginal tentacles lacking (Fig. 2A). Outer membranes of gonophores composed of two ectodermal layers (outer and inner ectoderm). Endoderm lining gastric cavity within radial and ring canals (outer endoderm) between two layers of ectoderm, and within a cylindrical spadix (inner endoderm) in the center of gonophores. A few eggs, light brown in colour, developing to planulae between inner ectoderm and inner endoderm (Fig. 2B). Length of spadix relative to gonophore length varied depending on maturity. Spadix reaching from proximal to distal end of gonophores having eggs, but reduced after eggs develop into planulae (Fig. 2A). Planulae club-shaped, with cilia (Fig. 2C). Measurements of mature gonophores and larvae as in Table 2.

## 3. Hydrorhizas

Hydrorhizas a stolon network covered with thin periderm, growing in the grooves of host shells. Spines absent on hydrorhizas.

# 4. Nematocysts

Three types of microbasic euryteles (small, medium, and large) and one type of desmonemes present (Fig. 3).

Large and medium microbasic euryteles found throughout the body of tentaculozooids and in

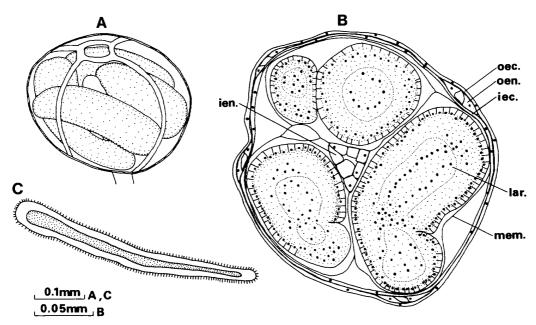


Fig. 2. Stylactaria multigranosi sp. nov. Gonophores and a larva (Holotype). A: Mature female gonophore with four radial canals and a ring canal contained four larvae; note the spadix does not reach the ceiling of the gonophore. B: transverse section of a gonophore (Fig.2A) with a single layer of outer ectoderm (oec.), four radial canals formed by outer endoderm (oen.), a single layer of inner ectoderm (iec.), and an inner endodermal spadix (ien.); note each larva (lar.) is covered with thin membrane (mem.); C: larva with cilia just after hatched out from a gonophore (Fig.1B).

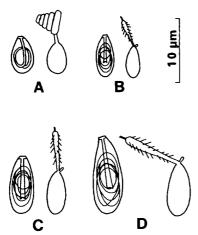


Fig. 3. Stylactaria multigranosi sp. nov. Nematocysts (Holotype). A: desmonemes from a tentacle of a gastrozooid; B: small microbasic euryteles from a larva; C: medium microbasic euryteles from a tentacle of a gastrozooid; D: large microbasic euryteles from a hypostome of a gastrozooid.

hypostome of gastrozooids and normal gonozooids. Desmonemes and medium microbasic euryteles on tentacles of gastrozooids and gonozooids. Medium microbasic euryteles on the surface of gonophores. Large and medium microbasic euryteles and desmonemes on body of each zooid.

Small microbasic euryteles present in planula larvae.

All types of nematocysts except for small microbasic euryteles present in stolon, although large microbasic euryteles confined to the tip.

Dimensions of each nematocyst type in holotype in Table 4.

Table 4. Stylactaria multigranosi sp. nov. Dimensions (mean  $\pm$  S.D., range) of each type of nematocysts for Holotype ( $\mu$ m)

	N	Length	Width
Desmonemes	30	6.0±0.4 (5.6-6.4)	$2.8\pm0.3$ $(2.4-3.2)$
Microbasic eur	yteles		
small	30	$6.8\pm0.4$ $(6.4-7.2)$	$2.4\pm0.4$ $(2.0-2.8)$
medium	30	$8.2\pm0.3$ $(8.0-8.8)$	$2.6\pm0.3$ (2.4-3.2)
large	30	$10.6\pm0.9$ (9.6-12.0)	$3.9\pm0.3$ (3.6-4.4)

N: number of nematocysts (desmonemes in the tentacles of 3 gastrozooids, small microbasic euryteles in 3 larvae, medium microbasic euryteles in the tentacles of 3 gastrozooids, large microbasic euryteles in hypostomes of 3 gastrozooids).

#### **DISCUSSION**

In addition to ten species previously assigned to Stylactaria [1, 4, 5], I adopt here new combination names for other species of the nominal genus Stylactis, i.e. Stylactaria hooperii (Sigerfoos, 1899), S. halecii (Hickson and Gravely, 1907), S. misakiensis (Iwasa, 1934), S. betkensis (Watson, 1978), S. sandrae (Wedler and Larson, 1986), S. spiralis (Hirohito, 1988), S. brachyurae (Hirohito, 1988), S. inabai (Hirohito, 1988), S. monoon (Hirohito, 1988), S. reticulata (Hirohito, 1988), S. spinipapillaris (Hirohito, 1988) [6–11]. I do not accept Calder's [1] view that S. hooperii is conspecific with S. arge, because S. hooperii differs clearly from S. arge in having no eggs developing into planulae within the gonophores [6, 12].

H. M. the Showa Emperor Hirohito [11] classified species of Stylactaria (=Stylactis) from Japan into five groups according to their gonophore types, i.e. short-lived medusae, eumedusoids, cryptomedusoids, heteromedusoids, and no gonophores (gonads developing in body wall of gonozooids). Recent works, however, revealed that S. conchicola and S. uchidai from Hokkaido, Japan, have styloid gonophores [4, 5]. Consequently, species of Stylactaria from Japan can be arranged in six groups according to styloid type, and other species of Stylactaria can be referred to one of these six groups (Table 5).

The present new species is assigned to Stylactaria by having polymorphic zooids and the stolon covered with perisarc. Stylactaria multigranosi differs from the syntopic species, S. conchicola and S. uchidai in having eumedusoid gonophores (Table 5), and is easily distinguished from five congeners with eumedusoid gonophores as follows: 1) the absence of marginal tentacles, 2) fixed gonophores, 3) eggs developing into planulae in the gonophore (Table 6).

Although the detailed morphology of the mature gonophores is unknown in two other species with eumedusoid gonophores, S. piscicola and S. sandrae (Table 6), S. multigranosi is distinguishable from these two species by the difference of their host species (Table 5). Stylactaria multigranosi resembles S. arctica, gonophores of which are unknown, in size of gastrozooids, but differs in

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TABLE 5. Species of the genus Stylactaria

Species	[]	Substrata	MHG	NT/NW	TG
S. multigranosi		Nassarius multigranosus	2.3	16/1	Eu
sp. nov.					
S. inermis	[13]	seaweed	4.2	20/2	
(Allman, 1872)	[14]	Barnacles, Fucus			Eu
S. arge	[12]	Zostera	45	30/2	SM
(Clarke, 1882)	[15]	Zostera marina, Entiromorpha sp., Bittium sp.	40	20/2	
	[ 1]	Cerithium litteratum			
S. hooperii	[ 6]	Ilyanassa obsoleta	25	30/1	SM
(Sigerfoos, 1899)					
S. arctica	[16]	Mohnia mohni	3	15/1	ND
(Jäderholm, 1902)					
S. halecii	[7]	Halecium arboreum	2	10/1	<del></del> -
(Hickson and Gravely, 1907)	[11]	Acanthopeltis japonica			
S. ingolfi	[17]	Homalophiura tesselata	2.5	17/1	Cr
(Kramp, 1932)		•		_	
S. piscicola	[18]	Erosa erosa	10	25/1	Eu
(Komai, 1932)				_	
S. misakiensis	[8]				
(Iwasa, 1934)	[11]	Living and/or hermit crab inhabited Gastropod shells	2	30/1	Eu
S. yerii	[19]	Turricula kamakurana	1.5	14/1	Cr
(Iwasa, 1934)	[11]	Pseudoetrema fortilirata	1.0	11/1	C.
S. carcinicola	[20]	Macrocheira kaempferi	10	30/1	SM
(Hiro, 1939)	[11]	Rock, Gastropod shells, Crustaceans		00.1	01.1
S. conchicola	[21]	,			
(Yamada, 1947)	[5]	Homalopoma amussitatum	2.3	$5/1^{1)}$	St
,		1	1.7	8/11)	
S. uchidai	[21]			0.1	
(Yamada, 1947)	[ 4]	Rock, seaweed, Sponge, Bivalves, Tubeworms Barnacles, Bryozoa	3.2	10/1	St
S. claviformis	[22]	Stone	16	32/2-3	Eu
(Bouillon, 1971)	( J	-		J = 1 = J	u
S. betkensis	[ 9]	Parcannassa burchardi	1.2	15/1	Cr
(Watson, 1978)	r . l			10,1	
S. sandrae	[10]	Solitary Tunnicate	2	16/1	Eu
(Wedler and Larson, 1986)	J	• · · · · · · · · · · · · · · · · · · ·	_	2011	u
S. spiralis	[11]	Small gastropod shells hermit crab inhabited	2	50/1	Cr
(Hirohito, 1988)	[]	Bassepa such normic cruo minuoted	_	50/1	Ci
S. brachyurae	[11]	Spider crab	1	14/1	Cr
(Hirohito, 1988)	[-+J	-F	•	17/1	CI
S. inabai	[11]	Small gastropod shells hermit crab inhabited	3	30/1-5	Eu
(Hirohito, 1988)	[]	5 6 Series normic vide milabited	,	50/1-5	Lu
S. monoon	[11]	Sponge, Endendrium	2	11/1	He
(Hirohito, 1988)	[**]	orongo, missionismis	2	11/1	116
S. reticulata	[11]	Rock, Barnacle, Bryozoa	5	12/1	Cr
(Hirohito, 1988)	[**]	Tion, Daniacio, Diyozoa	J	14/1	CI
S. spinipapillaris	[11]	Simplicifusus graciliformis	1.5	12/1	Eu
(Hirohito, 1988)	[**]	omprogram gracingorina	1.5	12/1	Eu
(12101110, 1700)					

<sup>[ ]:</sup> references; MHG: maximum height of gastrozooids (mm); NT: number of tentacles in gastrozooids; NW: number of tentacle whorls in gastrozooids; TG: type of gonophore; SM: short-lived medusae; Eu: eumedusoids; Cr: cryptomedusoids; He: heteromedusoids; St: styloids; —: gametes enclosed by a layer of ectoderm and situated in the body wall of one side of gonozooid; ND: no description.

<sup>1)</sup> Dimorphic gastrozooids (slender type and thick type) in S. conchicola.

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TABLE 6. Comparison of the characteristics of gonophores among *Stylactaria* species with eumedusoid gonophores

Species	Canals	Tentacles	Liberation of gonophores	Brooding of larvae
S. multigranosi sp. nov.	+	_	_	+
S. inermis	+	_	+	_
S. piscicola	+	ND	ND	ND
S. misakiensis	+	+	+	_
S. claviformis	+	+	+	_
S. sandrae	+	ND	ND	ND
S. inabai	+	+	+	_
S. spinipapillaris	+	+	+	

+: present; -: absent; ND: no descripition.

host species (Table 5). Because of their incomplete description, the difference in host species alone discriminates *S. multigranosi* from the above three species. Although such a host difference may be insufficient to identify *Stylactaria* species in general, I treat here *S. multigranosi* as a new species distinct from the above three species because *S. multigranosi* is specific in its preference of host gastropod as discussed later. Further information about gonophores of incompletely described species is needed both to accurately classify *Stylactaria* species and to demonstrate their phylogeny in accordance with the evolution of gonophores.

As in some species [5, 11], S. multigranosi shows intercolonial variation of zooid polymorphism. This species possesses four types of zooids, but it must be pointed out that a given colony does not have every type of zooid (Table 3). Two types, gastrozooids and normal gonozooids, were always

present, while non-hypostomed gonozooids were found in about 1/5 of the colonies examined and the tentaculozooids in about 1/100 of the colonies. The rare occurrence of tentaculozooids may show that this zooid type is aberrant in this species. Such rarity of tentaculozooids in colonies has been reported also in other *Stylactaria* species, namely *S. misakiensis*, *S. spiralis*, *S. inabai*, and *S. conchicola* [5, 11]. This seems to indicate that the presence of tentaculozooids is not a reliable way to identify *Stylactaria* species, although it has been adopted as a discriminating character in the key to *Stylactis* (=*Stylactaria*) from Japan [11].

Stylactaria multigranosi shows strong substrate preference, being found only on the gastropod Nassarius multigranosus. Many types of possible substrate were examined [4, 5], but none supported S. multigranosi. Therefore, it is concluded that S. multigranosi is specific in its preference of host species. The present species occurred on

TABLE 7. Association rates of Stylactaria multigranosi sp. nov. with the gastropod Nassarius multigranosus

	Total no.	Percentage of	No. of colonies collected			
	of shells examined	association (%)	Female	Male	Immature	
Sandy-mud bottom	ıs					
Oshoro	895	(15.5)	202	0	91	
Usu	224	(54.9)	104	0	19	
Total	2119	(19.6)	306	0	110	
Rocky shore						
Oshoro	571	(0.0)	0	0	0	

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about 1/5 of the specimens of N. multigranosus collected from sandy-mud bottoms, but on none collected from rocky shores (Table 7). The association rate of S. multigranosi with gastropods from sandy-mud bottoms is significantly different between Oshoro and Usu. More colonies occurred in Usu than in Oshoro (G test; G=157.79343, P<0.001). The association rate may vary depending on the habitat of host shells. No other sessile animals were found on N. multigranosus.

Parthenogenesis may occur in the present new species, because all the matured specimens collected were female (Table 7). Sexual reproduction in this new species will be investigated in my ongoing work.

#### **ACKNOWLEDGMENTS**

I wish to express cordial thanks to Dr. Dale R. Calder, Royal Ontario Museum, and Professor Shunsuke F. Mawatari, Hokkaido University, for their critical revision of the manuscript. Sincere gratitude is due to Dr. Shin Kubota, Hokkaido University, for his valuable advice. I am also indebted to Mr. Yasuhiro Kuwahara, Wakkanai Fisheries Experimental Station, for the identification of host gastropod species, and to Mr. Kazuro Shinta, Oshoro Marine Biological Station, Hokkaido University, for his kind assistance in collecting specimens.

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